Advantages and Context of Omic Approaches for Microbial Risk Assessments of Spacecraft Environments

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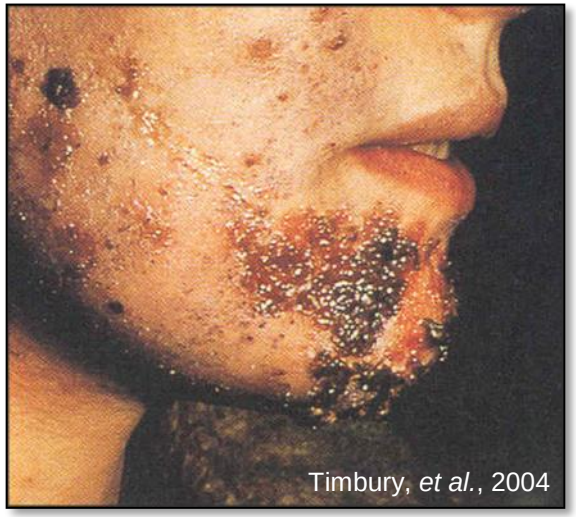
Why Monitor Spacecraft Environments

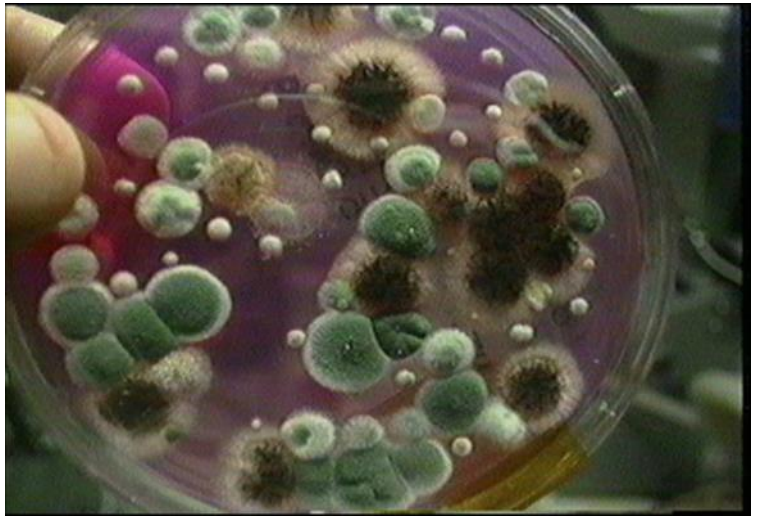


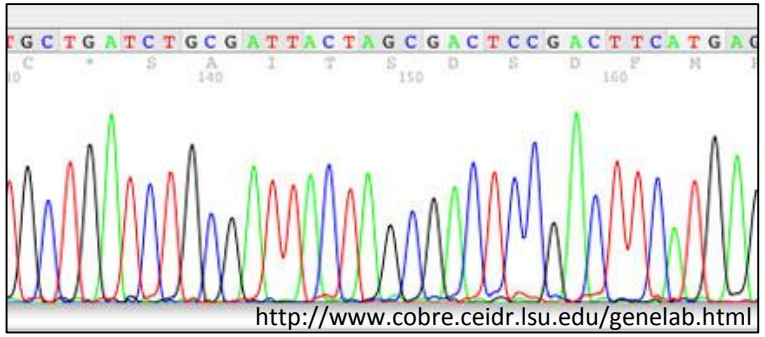
- Infectious Disease
- Systems failure
- Biodegradation
- Food spoilage
- Release of volatiles

Prevention

Current Microbiological Requirements

- Current spaceflight requirements are based on classical microbiological enumeration using “colony forming units” that reflect the number of microorganisms that grow on a media plate.
  - Example: Flight surface requirement for bacteria = 10,000 CFU/cm^2
- Current spaceflight requirements also include identification of medically significant organisms.
  - Identification originally relied on biochemical testing allowing us to describe the genus and species.
  - Technological advances have allowed us to identify microorganisms using DNA based tools.





Preflight Monitoring and Disinfection

- Possible routes of infection during spaceflight missions
  - Crew
  - Spaceflight food and water
    - *Salmonella enterica* serovar Typhimurium
    - *Staphylococcus aureus*
  - Vehicle air, surfaces, and cargo
    - *Pseudomonas aeruginosa*
    - *S. aureus*
  - Experimental Payloads (Biosafety Review)
    - *S. Typhimurium*
    - Methicillin resistant *S. aureus* (MRSA)
- Disinfection criteria
  - High microbial concentrations
  - Medically significant organisms

Contamination Potential

Preflight contamination

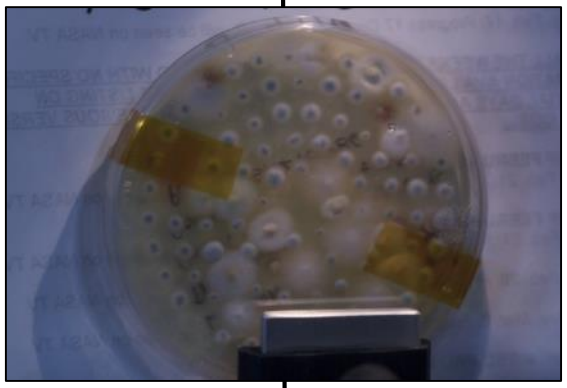
Spacecraft are complex

Astronaut activities
(*e.g.*, eating and hygiene)

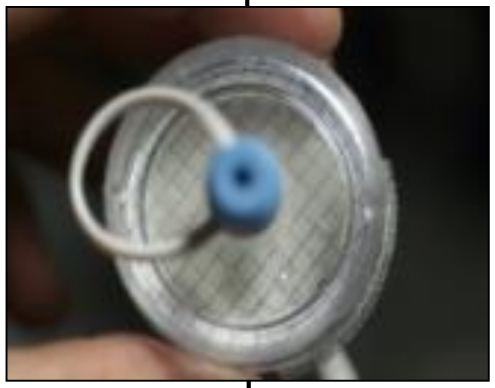
Microbiological Monitoring on the ISS

Surfaces

Air



Water



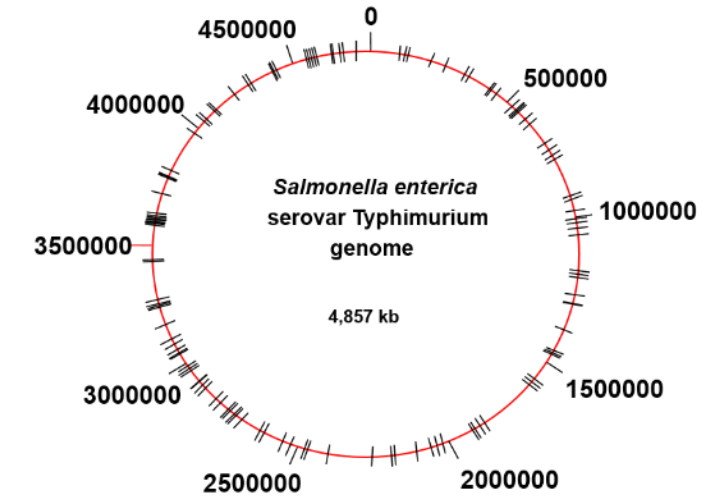
Quantified in-flight and returned to JSC for identification

Next Generation Spaceflight Monitoring

- Spaceflight technology demonstrations
  - Razor EX (Biofire Defense)
    - Targets selected microorganisms or groups of microorganisms
    - Real Time Polymerase Chain Reaction (RT-PCR)
    - Designed for and used by the military
    - Dry chemistry for easier sample prep
  - MinION (Oxford Nanopore)
    - Sequences all organisms in the sample
    - Nanopore technology
  - Both systems performed well in recent ISS technology demonstrations
- As these technologies do not enumerate microorganisms using CFUs, and the goal is autonomous environmental microbiological monitoring, new requirements are needed.

The Impact of the Spaceflight Environment on *Salmonella enterica* serovar Typhimurium

- In 2006, the MICROBE Experiment (PI: Cheryl Nickerson, Arizona State University) identified **alterations in microbial virulence** in spaceflight grown cultures of S. Typhimurium.
  - Proteomic profiling identified **73 differentially regulated proteins**, and microarray analysis identified **167 differentially regulated genes** compared to ground controls
  - Common molecular regulatory protein (Hfq) associated with 32% of the differentially regulated genes
  - Genes were globally distributed and associated with:
    - Protein secretion
    - Outer membrane proteins
    - Iron metabolism and storage
    - Ion response pathways
    - Plasmid transfer functions
    - Energy and metabolism
    - Ribosomal proteins
    - Small regulatory RNAs
    - Biofilm formation
    - Transcriptional regulators



Wilson *et al. Proc Natl Acad Sci USA* 2007

Developing New Requirements

- What information do we really need?
- As a starting point, we begin with information that is equivalent to our current approach.
  - Do we need less information based on lessons learned from the past?
  - Is there information that we did not require in the past that should be required in the future?
    - Toxin production
    - Ability to track a given microbial clone
- What impact will vehicle design have on the required information?

Developing New Requirements

- More devils in the details
  - How important is **enumeration**? If we do not use CFUs, then what would we use?
  - We want to **identify medically significant organisms**, but can we provide a list?
  - How will new technologies indicate microbial **viability**?
  - **Antibiotic resistance** is often important to know. How will autonomous spacecraft system provide this information?
- Engineering design of the monitoring system
  - What should be the **detection sensitivity** (*e.g.*, 1 bacterium per liter)?
  - How do we define the **required confidence** in the data (replicates, accuracy, depth, coverage)?
  - Should it include **software to analyze the data** (Green light-Red light)?

Moving forward

- The goal is to require monitoring approaches that provide the **best available information** to maintain both crew health and vehicle integrity.
- We can start out with an **equivalent information** approach, basically requiring that any new technology on the International Space Station provide information that meet our current standards.
- As we look to future missions, NASA will build requirements through **interactions** with internal and external experts in fields including computational biology, molecular biology, microbial pathogenesis, microbial risk assessment, infectious disease medicine, and hardware development.

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